Effects of Hexametaphosphate Levels on *Listeria innocua* Growth in Ready-to-Eat Meat Products

Honor's Project Report

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Abstract

U.S. meat processors are currently looking for intervention methods for preventing growth of *L. monocytogenes* during and after cooking of Ready-to-Eat (RTE) meat products. RTE meat products do not require further heat treatment before consumption and are often eaten directly out of the package. Phosphates, already a common ingredient in meat, have shown varying degrees of inhibition of different pathogens. At high concentrations, some phosphates have caused off-flavors in meat products. Due to its neutral pH, sodium hexametaphosphate (HMP) causes less flavor problems. Sodium hexametaphosphate has shown some inhibitory effectiveness with *S. typhimurium*, *S. aureus* and *C. botulinum*. With *L. monocytogenes*, only 0.5% HMP in agar has been studied; additional research needs to be conducted on the use of HMP and *Listeria* in actual meat products. The objective was to determine if HMP addition in fully-cooked meat products had an antimicrobial effect on *L. innocua* growth over time. The effect of phosphate concentration was also analyzed. Beef roasts were prepared using fresh beef, 0.5% sugar, 1.75% salt, water (30% brine solution), and sodium hexametaphosphate. Phosphate treatments included a control (no added phosphate), 0.5% and 1.0% sodium hexametaphosphate. Meat mixtures were cooked in a smokehouse to 170ºF internally and inoculated with *L. innocua*, serving as a surrogate to *L. monocytogenes*. Samples were plated on Modified Oxford Selective Agar initially and after 3, 6, and 9 days of refrigerated storage. Results showed no significant difference existed between replicates. However, there was a significant interaction between treatment and day. A significant difference between phosphate levels was not experienced until *L. innocua* had grown to Day 6. Data of growth from Day 9 graphically showed a clear trend between levels, but the data points were too spread out to be significant. Analyzing the effect over time, control and 0.5% HMP showed a significant difference, indicating that the microorganism grew during refrigerated storage. One percent phosphate was not significantly different, indicating a bacteriostatic effect on *Listeria innocua*. Implications of this are that 1% sodium hexametaphosphate can serve as a bacteriostatic agent against *L. innocua* growth in cooked meat products. The current limit of phosphate allowed by USDA FSIS is 0.5%, however, that level of phosphate showed no significant inhibitory effect. If further research confirms that concentrations of HMP greater than 0.5% contribute antimicrobial benefits, companies should petition the USDA FSIS to reassess critical limits for HMP given its added function in meat products because food safety is the number one issue facing the meat industry.

INTRODUCTION:

U.S. meat processors are currently looking for intervention methods for preventing growth of *Listeria monocytogenes* during and after cooking of Ready-to-Eat (RTE) meat products. These items do not require further heat treatment before consumption and thus are more frequently tested by the USDA FSIS and the company[1].

Phosphates, already a common ingredient in meat, have shown varying degrees of inhibition of different pathogens. The suggested antimicrobial mechanism of phosphate is its ability to bind divalent cations such as calcium, magnesium, and iron[2]. At higher concentrations (above 0.5%), some phosphates have caused off-flavors in meat products. Due to its neutral pH, sodium hexametaphosphate (HMP) causes less flavor problems[3]. Sodium hexametaphosphate has shown some inhibitory effectiveness with *Staphylococcus typhimurium, Staphylococcus aureus,* and *Clostridium botulinum*[4,5,6]. With *L. monocytogenes*, only 0.5% HMP in agar has been studied[7]. Additional research needs to be conducted on the use of HMP and *Listeria* in actual meat products. The objective was to determine if HMP addition in fully-cooked meat products had an antimicrobial effect on *L. innocua* growth over time. The effect of phosphate concentration was also analyzed.

MATERIALS AND METHODS:

Bacterial Culture and Media

The strain of *Listeria innocua* (ATCC 33090) obtained from the Food Safety Engineering Laboratory, Ohio State University, was purchased from the American Type Culture Collection (Manassas, Va.). *Listeria innocua* was cultivated in trypticase soy

broth supplemented with 0.1% yeast extract (TSBYE, Becton Dickinson-Difco, Spark, MD, USA) at 32 °C for 20 h prior to use. After cultivation, culture was harvested at 5,500 × g for 15 min at 4 °C (Sorvall® RC-5 PLUS; Thermo Electron Co., Kendro Laboratory, Asheville, NC, USA) and rinsed once with deionized water. The pellet was resuspended with sterile 0.1% peptone water (PW, Becton Dickinson-Difco). The harvested cells were diluted to approximately 10^8 CFU/ml in sterile 0.1% PW for inoculation.

Sample Preparation

Beef shoulders used in this research were obtained from the meat department at Kroger Grocery Store, Columbus, Ohio. Beef shoulders were ground using a ½ ” and 1/8“ grind plate. Brine solutions were prepared by first dissolving the sodium hexametaphosphate (Vitrofos®, Innofos, Cranbury, NJ) in water. The three treatment levels by weight basis included 0% (0 g), 0.5% (1.3 g), and 1.0% (2.6 g) sodium hexametaphosphate. Salt (4.55 g) and sugar (1.30 g) were then mixed into the solutions. Ground meat (200 g) and 30% brine solution (60 g total) were thoroughly homogenized with a spoon and vacuum packaged in plastic bags. Samples were cooked in an Alkar Oven (Lodi, WI) smokehouse until the internal temperature reached 77^{o}C for 15 minutes. After cooling to room temperature and mixing sealed meat bags by hand, 9.9 g samples with 0.1 g of *L. innocua* at approximately 10^8 CFU/g. were aseptically vacuum packaged in sterilized plastic bags and stored at 4 ^{o}C for 0, 3, 6, and 9 days. In previous studies, the storage time was 2 weeks-2 months with longer frequencies between plating. *Listeria innocua* colonies grew too quickly to note a difference between treatments so duration

was decreased to 9 days with more frequent plating. Because the product was cooked, 9 days is a realistic storage time for evaluating the meat samples.

Media

Modified Oxford Media (MOX; Difco, Detroit, MI, USA), 57 g, was dissolved with a magnetic spin bar in 1 liter of distilled water using a 1,000 ml Erlenmeyer flask. The flask was capped and autoclaved for 20 minutes at 121°C. Antibiotic supplement was dissolved in 10 mL of sterile distilled water and aseptically added to the solution upon cooling to 45°C; the magnetic spin bar further mixed the two liquids. Agar was poured into Petri dishes and stored in the refrigerator until use.

Microbial analysis

Samples (10 g) were aseptically mixed with 100 mL of sterile distilled water in a Whirl-Pak bag (Fisher Scientific, St. Louis, MO, USA). The mixtures were blended for 2 min in a stomacher laboratory mixer (Stomacher Model 400, Tekmar Co., Cincinnati, OH, USA). Mixtures were serially (1:10) diluted with 0.1% buffered peptone water (BPW). Sample dilutions (0.1 mL) were plated on the surface of selective Modified Oxford (MOX) agar. The agar plates were incubated to determine the populations of *L. innocua* at 32^{o}C for 48 h.

Data Analysis

Both replicates of *Listeria innocua* CFU's were analyzed with an Anova using P=0.05 as significant and P=0.01 as highly significant. The factors evaluated were the phosphate treatment and days of storage.

RESULTS AND DISCUSSION:

A significant interaction ($P < 0.05$) between phosphate treatment and day of storage existed, showing sodium hexametaphosphate had an effect on *Listeria innocua* growth (Figure 1). Over 9 days of storage, the control was highly significantly different ($P<0.01$) (Table 1) and 0.5% was significantly different ($P<0.05$) (Table 1), indicating that *Listeria innocua* grew in both treatments. However, the *Listeria innocua* in 1% phosphate treatment was not statistically different ($P>0.05$) (Table 1) during storage, indicating an inhibition of the microorganism. Phosphate levels at 0.5% may not bind sufficient metal ions (e.g., magnesium, iron, etc.), leaving enough to allow for microbial replication. Looking at specific days, inoculation levels were not significantly different at day 0

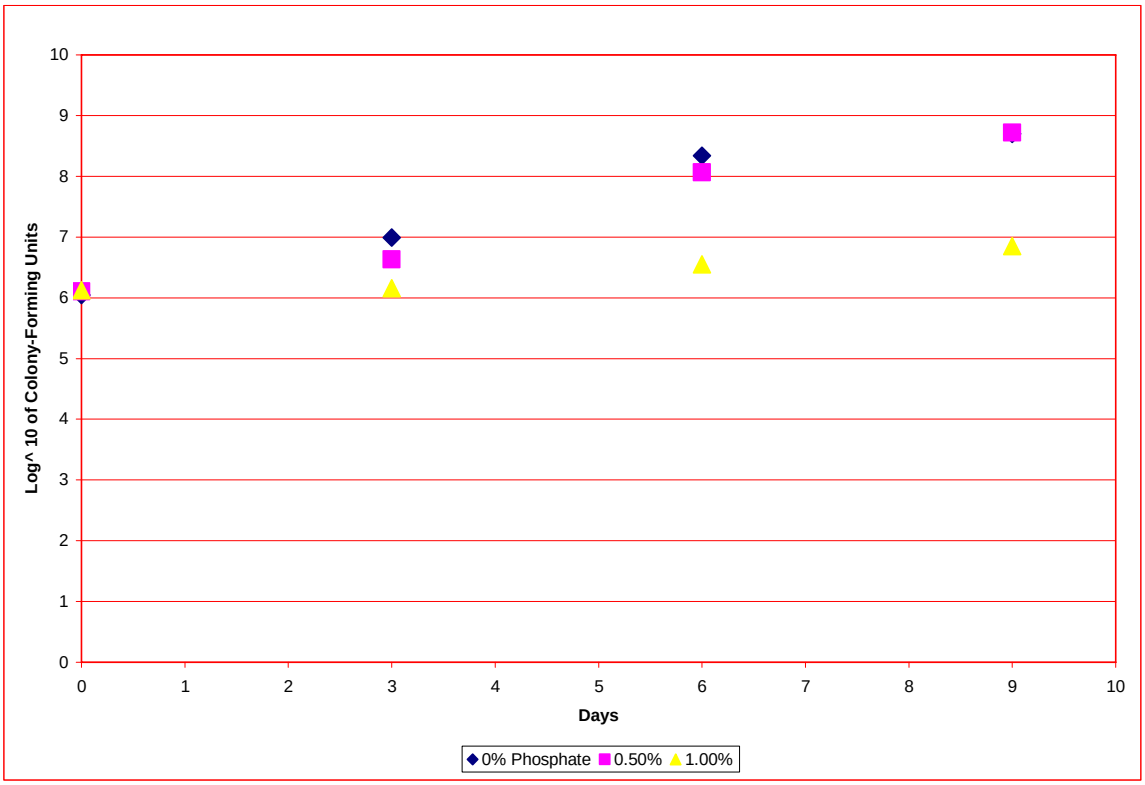

Figure 1. *Listeria innocua* growth in meat samples during refrigerated storage

Table 1. Mean treatment effects of phosphate treatment on *Listeria innocua* growth

Phosphate Treatment	P-value
Control	0.0009
0.5%	0.019
1.0%	0.68

($P < 0.05$) (Table 2, Fig 1). At day 3, the difference between phosphate treatments was approaching significance ($P=0.067$) (Table 2). A highly significant difference ($P<0.01$) (Table 2) existed at day 6, suggesting that hexametaphosphate may have antimicrobial properties at 1% concentrations during minimal storage but not at a 0.5% concentration. At day 9, no significant difference existed ($P>0.05$) (Table 2). This is likely due to large standard deviations with only 2 replicates because a clear linear trend of growth is apparent for 0% and 0.5% phosphate levels (Figure 1).

Table 2. Mean treatment effects of storage time on *Listeria innocua* growth

Storage Day	P-value
0	0.63
3	0.067
6	0.0001
9	0.11

CONCLUSIONS:

1. A significant interaction ($P < 0.05$) between hexametaphosphate levels and refrigerated storage time was found.
2. Results of the study suggest that hexametaphosphate may serve as a bacteriostatic agent against *Listeria monocytogenes* in ready-to-eat meat products. Phosphate levels at 1% appear to be bacteriostatic for *Listeria innocua,* whereas microbial

counts in meat containing 0.5% phosphate do not differ from the control. Additional replicates are needed.

3. A full-scale study using hexametaphosphate and *Listeria monocytogenes,* inoculated in cooked meat products, should be conducted to confirm these results. Based on the outcome, the 0.5% legal limit of phosphates in meat products may need to be reconsidered to allow higher levels of use in inspected meat products.
4. Possible flavor changes with higher concentrations of hexametaphosphate need to be evaluated with sensory testing to determine if negative taste perception exists.

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